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(54) Title: THERAPEUTIC AGENTS BASED ON MONOCLONAL ANTI-IDIOTYPIC ANTIBODY 105AD7

(57) Abstract

Substances based on monoclonal antibody 105AD7 are described, in fragments and CDRs of this antibody which are useful in generating an immune response against tumour associated antigens and have application in the therapeutic and prophylactic treatment of such tumours. Nucleic acid encoding the substances and uses of the substances and the nucleic acid are disclosed.

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THERAPEUTIC AGENTS BASED ON MONOCLONAL ANTI-IDIOTYPIC ANTIBODY 105AD7

Field of the Invention

The present invention provides substances which are useful in generating an immune response against tumour associated antigens and have application in the therapeutic and prophylactic treatment of such tumours, and to nucleic acid encoding these substances. The present invention further provides compositions, e.g. pharmaceutical compositions, comprising the above substances or nucleic acid, and uses of these substances in the treatment of conditions such as cancer.

Background of the Invention

EP-A-0440689 describes immunogenic compositions and methods for selectively treating cancer. These are based upon the production of human anti-idiotypic monoclonal antibodies which are capable of stimulating an anti-tumour response in an immunized patient.

A particular antibody claimed in EP-A-440689 is the human monoclonal anti-idiotypic antibody 105AD7. This antibody mimics T cell epitopes on the novel tumour associated antigen gp72 and has been developed as a therapeutic vaccine (Austin E.B. et al., Immunology, 67,525, 1989 and Austin E.B. et. al., J. Natl. Cancer Inst, 83,1245, 1991).

105AD7 has been tested extensively in a series of clinical trials. No antibody related toxicity was observed in any of the 163 patients entered into Phase I/II clinical trials of 105AD7, whereas both helper and cytotoxic T cell responses were induced. The helper responses were exemplified by induction of IL-2, antigen specific blastogenesis and enhanced NK activity. Anti-tumour cytotoxicity was measured directly and was supported by activation of circulating CD8 cells. Moreover, patients in the 105AD7 study had a brisk and significant lymphocytosis following immunisation

In the original Phase I clinical study in advanced colorectal cancer patients, a total of 35 immunisations of

105AD7 were given to 13 patients (Robins R.A. et al., Cancer Research, 51, 5425, 1991 and Denton G.W.L. et. al., Int. J. Cancer, 57, 10-14, 1994). 105AD7 induced antigen specific blastogenesis responses which showed a positive correlation with survival. This was supported by the observation that 105AD7 immunised patients had a 300% survival advantage when compared with a contemporary group of patients treated in the same centre.

Most cytolytic T cells express the CD8 antigen. Co-expression of the CD45 RA/RO isoforms further divides this group into active or memory (CD45RO) or naive (CD45RA) cytolytic T cells. A drop in the CD8 RA/RO ratio therefore signifies accumulation of activated or memory cytotoxic T cells. Analysis of pre- and post-treatment CD8RA/RO and CD4RA/RO ratios were followed during 21 cycles of 105AD7 immunisation in eight patients. There was a drop in the CD8RA/RO ratio in 15/21 cycles. Similar decreases in the ratio of circulating CD4RA/RO lymphocytes were observed except there were less circulating lymphocytes of the CD4RA phenotype prior to immunisation and therefore the drop was less marked. All patients but one receiving multiple injections of 105AD7 showed a drop in the CD8RA/RO ratio indicating that 3-4 injections were necessary to induce a sustained T cell response. The only patient who failed to respond to 105AD7 immunisation with a reduction in the ratio of CD8RA/RO was patient ID04 who had progressive disease and died 12 weeks post immunisation.

Due to the lack of toxicity associated with 105AD7 immunisation, permission was granted for a phase II adjuvant study (Durrant L.G. et al., Cancer Research, 54, 4837-4840, 1994). Colorectal cancer patients were immunised with 105AD7 prior to surgery and boosted post operatively. Evidence of autologous anti-tumour cytotoxicity was observed in 3/4 patients. Enhanced NK activity was observed in 7/13 patients and was of interest as these cells can kill tumour cells which do not express gp72 or have lost MHC expression and therefore compliment

CTL killing. A preliminary analysis of patients immunised with 105AD7 in the adjuvant trial and who have been followed for a minimum of 12 months showed a significant improvement in survival ($p>0.05$) compared to a contemporary group of stage matched patients treated at the same centre. Thus, this antibody clearly has significant therapeutic potential.

Summary of the Invention

In a first aspect, the present invention provides a nucleic acid molecule which encodes a substance or reagent comprising (a) a polypeptide having an amino acid sequence substantially as shown in figure 1 or (b) a fragment of said polypeptide which is capable of stimulating an immune response, or (c) a variant of said polypeptide (a) or fragment (b) which is capable of stimulating an immune response.

In further aspects, the present invention provides expression vectors comprising the above nucleic acid molecules, operably linked to control sequences to direct its expression, and host cells transformed with these vectors.

In a further aspect, the present invention provides a substance which is a polypeptide encoded by the above nucleic acid molecules.

In a further aspect, the present invention provides a pharmaceutical composition comprising one of more of the above substances in admixture with a pharmaceutically acceptable carrier.

In a further aspect, the present invention provides one or more of the above substances for use in a method of medical treatment.

In a further aspect, the present invention provides a use of one or more of the above substances in the preparation of a medicament for the treatment of cancer. Preferably, the use is in stimulating an antitumour response in an patient immunized with the polypeptide.

The cDNA sequence of 105AD7 has been resolved as described in example 1 hereinafter and the variable region is shown in attached figure 1. The constant region of the 105AD7 antibody has the well known and recognised structure of human antibodies. The cloning and sequencing was not a simple process.

Variable region primers based on consensus sequences of the major variable region families failed to PCR amplify 105AD7 heavy chain. Therefore, it was decided to design a specific primer based on the N-terminal amino acid sequence. However, as most of the known antibodies begin their sequences with glutamic acid (E) or glutamine (Q), the active amine residues are susceptible to modifications that can result in the formation of pyroglutamate. This molecule is unreactive in the Edman degradation reactions used in automated protein sequencing. N-terminal amino acid sequencing showed that this was true for 105AD7. To overcome this pyroglutamate amino-peptidase (Boehringer Mannheim) was used to remove the blocking residue.

Brief Description of the Drawings

The present invention will now be described by way of example with reference to the accompanying drawings in which:

Figure 1 shows the sequence of 105AD7 in which figure 1A shows the variable region of the 105AD7 heavy chain comprising the whole sequence from the 5' end combined with the RC of the sequence from the 3' end as well as the derived amino acid sequence, and figure 1B shows the 105AD7 Kappa chain cDNA sequence, again with the derived amino acid sequence;

Figure 2 shows the amino acid sequence of the third complementarity determining region (CDR3) of the heavy chain;

Figure 3 is a table showing the motifs present in the sequence of figure 2; and,

Figure 4 shows the proliferation response to the CDRH3 peptide of 105AD7.

Detailed Description

As mentioned above, the present invention provides a nucleic acid which encodes a reagent comprising (a) a polypeptide having an amino acid sequence substantially as shown in figure 1 or (b) a fragment of said polypeptide which is capable of stimulating an immune response, or (c) a variant of said polypeptide (a) or fragment (b) which is capable of stimulating an immune response.

The polypeptides may additional comprise antibody constant domains, suitably human antibody constant domains. In such cases, the nucleic acids will additionally comprise sequences which encode said constant domains.

The above-mentioned variants are suitably polypeptides which show homology with the polypeptide of figure 1 or fragments thereof. Suitably they are show a degree of homology which is at least 60%, preferably at least 70%, more preferably at least 80%, more preferably 90% and still more preferably 95% to the polypeptide of figure 1. In general, they will be encoded by a nucleic acid which hybridises with the nucleic acid of figure 1 under stringent hybridisation conditions. Such conditions are conventional in the art and include for example those defined in Sambrook, Fritsch and Maniatis, "Molecular Cloning, A Laboratory Manual", Cold Spring Harbor Laboratory Press, 1989, and Ausubel et al, "Short Protocols in Molecular Biology", John Wiley and Sons, 1992.

The above nucleic acid can be readily prepared by the skilled person using the information and references contained herein and techniques known in the art. These techniques include (i) the use of the polymerase chain reaction (PCR) to amplify samples of such nucleic acid, (ii) chemical synthesis, or (iii) preparing cDNA sequences.

In order to obtain expression of the nucleic acid sequences, the sequences can be incorporated in a vector

having control sequences operably linked to the nucleic acid to control its expression. The vectors may include other sequences such as promoters or enhancers to drive the expression of the inserted nucleic acid, nucleic acid sequences so that the polypeptide is produced as a fusion and/or nucleic acid encoding secretion signals so that the polypeptide produced in the host cell is secreted from the cell. The polypeptide can then be obtained by transforming the vectors into host cells in which the vector is functional, culturing the host cells so that the polypeptide is produced and recovering the polypeptide from the host cells or the surrounding medium. Prokaryotic and eukaryotic cells are used for this purpose in the art, including strains of E. coli, yeast, and eukaryotic cells such as COS or CHO cells. The choice of host cell can be used to control the properties of the polypeptide expressed in those cells, e.g. controlling where the polypeptide is deposited in the host cells or affecting properties such as its glycosylation.

In a preferred embodiment, the nucleic acid of the invention comprises the sequence shown in figure 1 or a fragment thereof.

Particular fragments of interest are those which comprise or are derived from the idiotypic region of the polypeptide of figure 1. In particular, fragments comprising the complete variable region of 105AD7 or variants thereof may be utilised. This is because lymphocytosis is observed in patients receiving 105AD7 therapy and this response is thought to be dependent upon *in vivo* presentation of the complete variable region of 105AD7. The lymphocytosis is believed to provide the correct milieu of cytokines to stimulate the antigen specific helper and cytotoxic T cell responses.

It is possible that the reagent will stimulate immune responses to other antigens if it is co-injected by stimulating lymphocytosis and cytokine secretion.

However, smaller fragments and in particular those

encoded by the CDR3 region of the heavy chain have useful activity as will be described more fully below.

The sequence of figure 1 was analysed for T cell epitopes using an algorithm which predicts areas which contain amphipathic sequences, Rothbard motifs and DR and DQ binding peptides. The results of this show the following:

The variable region of 105AD7 heavy chain as shown in figure 1A comprises:

Leader sequence : nucleotides 3-->53
Mature peptide : nucleotides 54-->437
FR1 : nucleotides 54 -->143
FR2 : nucleotides 165 -->206
FR3 : nucleotides 255 -->344
CDR1 : nucleotides 144-->164
CDR2 : nucleotides 207-->254
CDR3 : nucleotides 351-->398
JR : nucleotides 387-->422
C1 : nucleotides 423-->

The entire region from positions 3 to 434 is translated.

The cDNA sequence of the 105AD7 Kappa chain shown in figure 1B comprises:

FR1 : nucleotides 1 --> 81
FR2 : nucleotides 115 -->159
FR3 : nucleotides 181 -->276
CDR1 : nucleotides 82-->114
CDR2 : nucleotides 160-->180
CDR3 : nucleotides 277-->303
JR : nucleotides 304-->

The entire region from 1 to 391 is translated.

A number of CDR regions have been identified and are shown in figures 1A and 1B. Particularly suitable

substances or reagents of the invention are those which comprise fragments including one or more of the CDR regions identified in figures 1A and/or 1B as well as active variants thereof. Such variants may include polypeptides comprising more than one such CDR region joined together. Other sequence variants having sequences differing from that given in figures 1A and/or 1B by one or more of addition, substitution, deletion and insertion of one or more amino acids are also included. Typically, these variants have at least about 60% sequence identity, preferably at least about 70% sequence identity, more preferably at least about 80% sequence identity, more preferably at least about 90% sequence identity, more preferably at least about 95% sequence identity with the sequences of figures 1A and/or 1B, and have the property of stimulating T cell responses in cancer patients in a similar manner to the whole 105AD7 anti-idiotypic antibody.

The third complementarity determining region (CDR3) of the heavy chain was identified as a 16 amino acid sequence, shown as figure 2 hereinafter. This region, which is the most variable region contained both amphipathic sequences and a Rothbard motif. Comparison of the CDRH3 region with over 100 class I and II binding motifs derived from a database of 4,000 T cell epitopes identified overlapping sequences which contained HLA/A1, HLA/A3, HLA/24, HLA/DR1, HLA/DR3, HLA/DR7 and two more promiscuous DR epitopes as illustrated in figure 3 hereinafter.

Cytotoxic T cell responses were measured in patients following 105AD7 injection. A response was defined as either a drop in the CD8 RA/RO ratio (activation) or as NK unrelated anti-tumour cytotoxicity. All of the patients who responded (10/12) expressed the HLA/A1, A3 or A24 haplotypes predicted from the 105AD7 epitope analysis.

The HLA/DR haplotypes and helper responses of 105AD7 patients were also analysed. All of the 10/13 patients who responded in either of the helper assays (antigen specific blastogenesis or induction of IL-2) were of the HLA/DR

1,3,7 phenotype with the exception of one patient who was HLA/DR2,6. Although the sequence of CDRH3 region does not have a HLA/DR2 motif, no HLA/DR6 motifs were available for analysis, so it could be possible that this haplotype could also be stimulated perhaps by one of the two promiscuous HLA/DR epitopes present in the CDRH3 of 105AD7.

These results show that in 10/12 (83%) patients receiving 105AD7 immunisation helper and cytolytic T cell responses were stimulated and all of these patients had haplotypes which were compatible with T cell motifs found in a 16 amino acid sequence of the CDRH3 (hypervariable) region of the anti-idiotypic antibody.

It appears therefore that the CDRH3 region would stimulate similar T cell responses in cancer patients in a similar manner to the whole 105AD7 anti-idiotypic antibody and thus this forms a preferred embodiment of the invention. The biological activity of the CDRH3 region is confirmed in examples 2 and 3 below.

The present invention provides a substance comprising a peptide comprising at least a part of the amino acid sequence of figure 2 or a functional equivalent or mimetic thereof, which reagent is capable of stimulating an immune response to a tumour antigen.

In general, the tumour antigen will be the gp72 antigen although in view of the promiscuous nature of some of the helper epitopes, it is possible that the reagents will stimulate immune responses to other tumour associated antigens. In fact, the helper responses could be expected to "help" CTL responses to any antigen if they are co-injected, and therefore the reagents of the invention have wide application in therapy and in vaccine technology.

The resolution of the sequence of 105AD7 allows the production of the antibody or immunologically active fragments or variants thereof using recombinant DNA technology.

Thus, the invention provides a method for preparing a substance which is capable of stimulating an immune

response, said method comprising incorporating a nucleic acid as described above in an expression vector, transforming a host cell with said expression vector, culturing said cell and recovering the product expressed by said nucleic acid.

Polypeptides obtainable by expression of the nucleic acids of the invention other than the 105AD7 antibody itself, forms a further aspect of the invention. The polypeptides of the invention may also be generated wholly or in part by chemical synthesis.

The substance will be capable of stimulating an immune response in a mammalian subject, in particular a human. The presence of such a response may be assessed using routine methods, for example those described in EP-A-440689 or mentioned hereinbefore.

Suitably the peptide comprises from four to sixteen and more suitably from six to sixteen consecutive amino acids of the figure 2 (VLYYDFWSGYLEYFAY) Preferably the peptide comprises at least one complete motif as illustrated in figure 3.

In a preferred embodiment, the peptide comprises the amino acid sequence of figure 2.

As used herein, the term "functional equivalent" refers to peptides which although differing in amino acid sequence, fulfill an essentially similar function in stimulating an immune response. It is understood that some amino acids in a peptide may be replaced by others without eliminating or substantially altering the activity of the peptide.

The polypeptides of the present invention may be used in screening for molecules having a similar activity. It is well known that pharmaceutical research leading to the identification of a new drug may involve the screening of very large numbers of candidate substances, both before and even after a lead compound has been found. This is one factor which makes pharmaceutical research very expensive and time-consuming. Means for assisting in the screening

process can have considerable commercial importance and utility. Such means for screening for substances potentially useful in treating or preventing cancer is provided by polypeptides according to the present invention.

Accordingly, in a further aspect, the present invention provides the use of at least a part of the amino acid sequence of figure 2 in a method of screening for functional equivalents or mimetics to this peptide. Preferably, this method includes testing candidate compounds for the property of stimulating an immune response to a tumour antigen.

The present invention also includes the use of one of the above peptide motifs in the design of an organic compound which is modelled to resemble the three dimensional structure of the peptide motif, the organic compounds having the property of stimulating an anti-tumour response in a patient. This is a step in finding mimetics to the polypeptides of the invention.

The term "mimetic" relates to compounds which are designed to mimic the peptide of the invention using approaches known in the pharmaceutical art. This might be desirable where the active compound is difficult or expensive to synthesise or where it is unsuitable for a particular method of administration. Mimetic design, synthesis and testing is generally used to avoid randomly screening large number of molecules for a target property.

There are several steps commonly taken in the design of a mimetic from a compound having a given target property in this case, the stimulation of an immune response to a tumour antigen. Firstly, the particular parts of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done by systematically varying the amino acid residues in the peptide, e.g. by substituting each residue in turn. These parts or residues constituting

the active region of the compound are known as its "pharmacophore".

Once the pharmacophore has been found, its structure is modelled according to its physical properties, eg stereochemistry, bonding, size and/or charge, using data from a range of sources, e.g. spectroscopic techniques, X-ray diffraction data and NMR. Computational analysis, similarity mapping (which models the charge and/or volume of a pharmacophore, rather than the bonding between atoms) and other techniques can be used in this modelling process.

In a variant of this approach, the three-dimensional structure of the ligand and its binding partner are modelled. This can be especially useful where the ligand and/or binding partner change conformation on binding, allowing the model to take account of this in the design of the mimetic.

A template molecule is then selected onto which chemical groups which mimic the pharmacophore can be grafted. The template molecule and the chemical groups grafted on to it can conveniently be selected so that the mimetic is easy to synthesise, is likely to be pharmacologically acceptable, and does not degrade *in vivo*, while retaining the biological activity of the lead compound. The mimetic or mimetics found by this approach can then be screened to see whether they have the target property, or to what extent they exhibit it. Further optimisation or modification can then be carried out to arrive at one or more final mimetics for *in vivo* or clinical testing.

It is also possible to take fragments or CDRs of 105AD7 disclosed herein and use the techniques of recombinant DNA technology to produce other antibodies or chimeric molecules which retain the specificity of the original fragment or CDR. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an antibody to the constant regions, or constant regions

plus framework regions, of a different immunoglobulin. See, for instance, EP-A-184187, GB-A-2188638A or EP-A-239400. Chimeric molecules comprising an immunoglobulin binding domain, or equivalent, fused to another polypeptide are therefore included. Cloning and expression of chimeric antibodies are described in EP-A-0120694 and EP-A-0125023.

It may also be desirable to use the CDR "humanise" non-human (e.g. murine) antibodies to provide antibodies having the antigen binding properties of the non-human antibody, while minimising the immunogenic response of the antibodies, e.g. when they are used in human therapy. Thus, humanised antibodies comprise framework regions derived from human immunoglobulins (acceptor antibody) in which residues from one or more complementary determining regions (CDRs) are replaced by residues from CDRs of a non-human species (donor antibody) such as mouse, rat or rabbit antibody having the desired properties, e.g. specificity, affinity or capacity. Some of the framework residues of the human antibody may also be replaced by corresponding non-human residues, or by residues not present in either donor or acceptor antibodies. These modifications are made to the further refine and optimise the properties of the antibody.

Various methods of administration of the reagent of the invention can be used, following known formulations and procedures.

Suitably an immunogenic or therapeutic amount of the reagent of the invention is administered to a patient.

An immunogenic or therapeutic amount is an amount which stimulates an immune response of a humoral and/or cellular nature in an immunized host, whereby the host's immune system exhibits increased activity against tumor cells bearing in particular the gp72 antigen. Thus the compositions have a therapeutic or protective effect in the afflicted host, in that the tumor cells are eliminated or partially arrested.

The reagents may be administered directly in a pharmaceutical composition or alternatively, where the reagents are peptides, they could be produced *in vivo* by expression from an encoding gene introduced into the system, e.g. in a viral vector. Thus in one embodiment, the invention provides a method of stimulating an immune response to a tumour antigen which comprises administration to a patient of an expression vector which comprises a nucleic acid of the invention.

Vectors such as viral vectors have been used in the prior art to introduce genes into a wide variety of different target cells. Typically, the vectors are exposed to the target cells so that transfection can take place in a sufficient proportion of the cells to provide a useful therapeutic or prophylactic effect from the expression of the desired polypeptide. The transfected nucleic acid may be permanently incorporated into the genome of each of the targeted tumour cells, providing long lasting effect, or alternatively the treatment may have to be repeated periodically.

A variety of vectors, both viral vectors and plasmid vectors, are known in the art, see US Patent No. 5,252,479 and WO 93/07282. In particular, a number of viruses have been used as gene transfer vectors, including papovaviruses, such as SV40, vaccinia virus, herpesviruses, including HSV and EBV, and retroviruses. Many gene therapy protocols in the prior art have used disabled murine retroviruses.

As an alternative to the use of viral vectors other known methods of introducing nucleic acid into cells includes electroporation, calcium phosphate co-precipitation, mechanical techniques such as microinjection, transfer mediated by liposomes and direct DNA uptake and receptor-mediated DNA transfer.

Adjuvants may be employed in the pharmaceutical compositions to facilitate stimulation of the host's immune response, and may include, but are not limited to, aluminum

hydroxide, lysolecithin, pluronic polyols, polyanions, peptides, proteins, oil emulsions and BCG. The reagents of the invention may also be coupled to immunogenic carriers or cross-linked. Physiologically-accepted carriers such as saline, sterile water, phosphate buffered saline, and the like, may also be used in the compositions. Other buffering and dispersing agents and inert non-toxic substances suitable for delivery to a patient may be incorporated in the pharmaceutical compositions and are well known to those skilled in the art. The solutions for administration are sterile and generally free of particulate matter. The compositions may be sterilized by conventional sterilization techniques. The concentration of reagent in the formulations can vary widely, i.e. from less than about 0.01%, usually at least 0.5%, to as much as 15 to 20% be weight and will be selected primarily based on fluid volumes, viscosities, antigenicity, etc., in accordance with the particular mode of administration selected.

The reagents of this invention are suitably formulated for parenteral administration, i.e. intravenously, intramuscularly, or subcutaneously. The reagents are administered to a patient diagnosed as having or suspected of having a tumor which bears the gp72 antigen, or may be administered prophylactically to a person predisposed to such disease. By "bearing gp72 antigen" is meant tumor cells which themselves express the gp72 antigen, or tumor cells to which the gp72 antigen has otherwise become bound or associated. The gp72 antigen is desirably accessible or may be made accessible to the antibodies or cells responding to the administration of the anti-idiotypic antibodies of the invention.

The reagents are administered in amounts sufficient to stimulate an immune response so that tumour cells are eliminated or their growth totally or partially inhibited. Amounts effective for this use will depend upon the severity of the disease and the status of the patient's

immune system, but generally range from about 0.1 μg to about 1 mg of reagent per kilogram of body weight, with dosages of 1 μg to 200 μg per kilogram being more commonly used. Administration may be once or a plurality of times, for instance at least three or four times, over a prolonged period. There may be no upper limit on the number of administrations possible, subject to the appearance of side effects. As the materials of this invention may be employed in serious disease states, that is, life-threatening situations, substantial excesses of these reagents may be administered if desired by the treating physician in view of the absence of extraneous substances and the absence of "foreign substance" rejection in a human host. Actual methods of preparing and administering pharmaceutical compositions, including preferred dilution techniques for injections of the present compositions, are well known or will be apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 16th Ed., Mack Publishing Co., Penn (1982).

The reagents of the invention may also be used in combination with other antibodies, which are preferably human, or may be given in conjunction with other chemotherapeutic agents such as doxorubicin, cisplatin and taxol. If the additional antibodies employed bind the gp72 antigen, they desirably do not bind the reagents also administered. To achieve possible synergistic action between the components the additional antibodies may bind determinants of gp72 which are not recognized by the immune response engendered by the reagents of the invention which are also administered. The additional monoclonal antibodies may be administered separately or in conjunction with the reagents of this invention, which may themselves be conjugated to chemotherapeutic agents. For instance, U.S Patent Nos. 4,590,071 and 4,671,958, describe the coupling of ricin A chain to a monoclonal antibody for targeting to human tumor cells.

Methods for the therapeutic or prophylactic treatment of tumours comprising administration of a reagent as described above also form part of the invention.

The following examples are provided to illustrates the invention and should not be construed as limiting its scope.

Example 1

Blotting onto PVDF

Purified 105AD7 was electrophoresed on a reducing 12% SDS PAGE gel and blotted onto PVDF; The PVDF was pre-wetted in methanol then both the gel and the PVDF were equilibrated in transfer buffer for 10 minutes. The transfer buffer used was; 10mM CAPS (cyclohexyleamino propane suphonic acid). pH 9.5-11.0 with NaOH (pH 10), 0.1% SDS, 10% methanol.

The presence of 0.1% SDS was essential for complete transfer of the antibody from the gel to the PVDF. Absence of this modification resulted in approximately 90% of the protein remaining in the gel. The transfer on a semi dry blotter was carried out in 3-4 hours at 120mA.

Following transfer the blot was rinsed in methanol washed in distilled water, stained with 0.2% Ponceau S in 1% acetic acid and destained in water. The heavy chain bands identified were excised from the blot and treated with pyroglutamate aminopeptidase. The excised band was then washed in distilled water and used in an automated protein sequencing reaction.

The results from the sequencing were sufficient to identify the variable region gene family used by the heavy chain:

Residue	1 Removed by peptidase
	2 no clear indication (V or P)
	3 no clear indication (P)
	4 appeared to be a **Leucine (L)**
	5 appeared to be a **Lysine (K)**

6 appeared to be a Glutamate (E)
7 no clear indication

This sequence **LKE** if compared to the known mature human heavy chain sequences is only present in the family derived from VH2 gene usage. The associated leader sequence used by the VH2 gene family was used to design an oligonucleotide primer that would enable us to amplify the cDNA heavy chain.

The variable region of 105AD7 heavy chain was PCR amplified using the VH2 primer, and HCI constant region primer. The PCR produced was cloned in to SK-vector and sequenced using the M13 universal and reverse sequencing primers. CDRs were defined by comparison of the 105AD7 sequence with a database of human antibody variable region sequences.

The light chain variable region was amplified using standard variable region primers based on consensus sequences of the major variable region families.

The PCR product was cloned using the M13 universal and reverse sequencing primers. CDRs were defined by comparison of this sequence with a database of human antibody variable region sequences.

Example 2

T cell proliferation responses

Peripheral blood lymphocytes from normal donors can be isolated from whole blood on ficol hypaque. Any lymphocytes which have been activated *in vivo* are then removed by a monoclonal antibody which recognises the CD45 RO activation marker which is linked to magnetic beads and the marked cells are removed by magnetic separation. The remaining lymphocytes can be stimulated with 105AD7 but not a control human IgG1 antibody. Figure 4 shows the growth of naive human lymphocytes which have been incubated at 37°C for 10-14 days and then DNA incorporation quantified for 16hrs by measuring incorporation of ^{3}[H]-thymidine. In an

identical assay a sixteen amino acid peptide which has the same amino acid composition as the CDR-H3 region of the 105AD7 monoclonal antibody can also stimulate lymphocyte proliferation (figure 4). Furthermore, if naive lymphocytes are stimulated with the 16 amino acid CDR-H3 peptide for 14 days and then restimulated with either the same peptide, an irrelevant peptide, 105AD7 or control human IgG1, the CDR-H3 primed lymphocytes respond significantly better to both the CDR-H3 peptide and 105AD7 than to control peptide or antibody (table 1).

Table 1

Priming of naive lymphocytes with CDRH3 peptide and challenge with the same peptide an irrelevant peptide control or 105AD7 or human IgG1. Proliferation is measured by ^{3}[H]-thymidine incorporation during the last 16hrs of the incubation and is expressed as cpm.

1st stimulant	2nd stimulant	Experiment 1 cpm ∓ sd	Experiment 2 cpm ∓ sd
CDRH3	CDRH3	4783 ∓ 265	22,844 ∓ 1892
CDRH3	control peptide	1918 ∓ 95	7,000 ∓ 1344
CDRH3	105AD7	3273 ∓ 304	10,725 ∓ 1393
CDRH3	hIgG1		7,161

Example 3

MHC binding

T-cells do not recognise native proteins, instead T cell receptors engage with small peptides derived by proteolytic digestion of proteins which are bound to Major Histocompatibility Molecules (MHC). The ability of these peptides to bind to MHC can be assessed in cell lines which are defective in proteolytic digestion and therefore produce empty unstable MHC molecules. If these cell lines are incubated with peptides which can bind to MHC they stabilise the expression of these molecules at the cell surface. This can be quantified by indirect

immunofluorescence staining with monoclonal antibodies which recognise MHC molecules. Peptides which have the amino acid sequences of the HLA-A3 motifs described in Figure 3 have been shown to stabilise the expression of the HLA-A3 molecule in the appropriate cell line.

Table 2

Expression of MHC on T2-A3 cell line in the presence or absence of MHC binding peptides.

	Expression of MHC (FR) b		
	1μM	10μM	100μM
No peptide			
HLA-A3 (5-13)a	1.8	1.3	1.3
HLA-A3 (7-16)a	and	1.5	1.9
HLA-A3 (1-10)a	1.2	1.8	1.8

a) peptides were based on the amino acid composition of CDR-H3 position 5-13 and positions 7-16.

b) MHC was stained by indirect immunofluorescence with the monoclonal antibody w6/32 which recognises a polymorphic determinant on HLA molecules. Expression was quantified by a flow cytometer and expressed as fluorescence ratio of peptide binding with and without w6/32 Mab. Significant binding is shown by a ratio greater than 1.2.

The references mentioned herein are all incorporated by reference in their entirety.

Claims:

1. A nucleic acid molecule which encodes a reagent comprising (a) a polypeptide having an amino acid sequence substantially as shown in figure 1 or (b) a fragment of said polypeptide which is capable of stimulating an immune response, or (c) a variant of said polypeptide (a) or fragment (b) which is capable of stimulating an immune response.

2. The nucleic acid molecule of claim 1 which encodes a complementarity determining region (CDR) shown in figure 1.

3. The nucleic acid molecule of claim 2 wherein the CDR is located between nucleotides 144-164, 207-254 or 351-398 of figure 1A or nucleotides 82-114, 160-180 or 277-303 of figure 1B.

4. The nucleic acid of any one of the claims 1 to 3 which encodes CDRH3 having the sequence shown in figure 2, or a fragment which is capable of stimulating an immune response.

5. The nucleic acid of claim 4 wherein the fragment encodes a peptide motif shown in figure 3.

6. An expression vector comprising a nucleic acid molecule of any one of claim 1 to 5, operably linked to control sequences to direct its expression.

7. A host cell transformed with a vector according to claim 6.

8. A substance which is a polypeptide encoded by a nucleic acid molecule of any one of claims 1 to 5.

9. A substance comprising a peptide comprising at least a part of the amino acid sequence of figure 2 or a functional equivalent or mimetic thereof, which reagent is capable of stimulating an immune response to a tumour antigen.

10. A method of producing a polypeptide of claim 8 or claim 9 comprising culturing the host cells of claim 7 so that the polypeptide is produced and recovering the polypeptide from the host cells or the surrounding medium.

11. A method of producing a polypeptide of claim 8 or claim 9 using chemical synthesis.

12. Use of the amino acid sequence of figure 2 or a functional fragment thereof in a method of screening for functional equivalents or mimetics to this peptide, the use including testing the functional equivalents or mimetics for the property of stimulating an immune response to a tumour antigen

13. A pharmaceutical composition comprising a polypeptide of claim 8 or claim 9 in admixture with a pharmaceutically acceptable carrier.

14. The pharmaceutical composition of claim 13 further comprising a chemotherapeutic agent.

15. The pharmaceutical composition of claim 13 or claim 14 wherein the chemotherapeutic agent is doxorubicin, cisplatin and taxol.

16. The polypeptide of claim 8 or claim 9 for use in a method of medical treatment.

17. Use of a polypeptide of claim 8 or claim 9 in the preparation of a medicament for the treatment of cancer.

18. The use of claim 17 wherein the use in stimulating an antitumour response in an patient immunized with the polypeptide.

19. A method of stimulating an immune response which comprises administering an effective amount of a polypeptide of claim 8 or claim 9.

20. A pharmaceutical composition comprising an expression vector of claim 6 in admixture with a pharmaceutically acceptable carrier.

21. The method of stimulating an immune response to a tumour antigen which comprises administration to a patient of an expression vector of claim 6.

```
Frame 3   D   T   L   C   Y   T   L   L   L   T   I   P   S   R   V   L   S
        GAC ACA CTT TGC TAC ACG CTC CTG CTG ACC ATC CCT TCA CGG GTC TTG TCC
                  11          20          29          38          47

          Q   V   T   L   K   E   S   G   P   T   L   V   K   P   T   Q   T   L   T
        CAG GTC ACC TTG AAG GAG TCT GGT CCT ACG CTG GTG AAA CCC ACA CAG ACC CTC ACG
                  62          71          80          89          98          107

                                                  <-------- (CDR H1) -------->
          L   T   C   T   L   S   G   F   S   L   N   T   S   G   V   C   G   V   W
        CTG ACC TGC ACC TTG TCT GGA TTC TCA CTC AAC ACT AGT GGA GTG TGT GTG GGC TGG
                  119         128         137         146         155         164

                                                                  <-------------------
          I   R   Q   P   P   G   K   A   L   E   W   L   A   H   I   Y   W   D   D
        ATC CGT CAG CCC CCA GGC AAG GCC CTG GAG TGG CTT GCA CAC ATT TAT TGG GAT GAT
                  176         185         194         203         212         221

- (CDR H2) ---------------------------->
          D   K   R   Y   S   P   S   L   K   S   R   L   T   I   T   K   D   T   S
        GAT AAG CGC TAC AGC CCA TCT CTG AAG AGC AGG CTC ACC ATC ACC AAG GAC ACT TCC
                  233         242         251         260         269         278

          K   N   Q   V   V   L   T   M   T   N   M   D   P   V   D   T   A
        AAA AAC CAG GTG GTC CTG ACA ATG ACC AAC ATG GAC CCT GTG GAC ACA GCC
                  290         299         308         317         326

                                        <---------- (CDR H3) ----------
          T   Y   Y   C   A   Q   V   L   Y   Y   D   F   W   S   G   Y   L   E   Y
        ACA TAT TAC TGT GCA CAG GTG CTG TAT TAC GAT TTT TGG AGT GGT TAT CTT GAA TAC
        335         344         353         362         371         380         389

---------->
          F   A   Y   W   G   Q   G   T   L   V   T   V   S   S   A   S   T   K   G
        TTT GCC TAC TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA GCC TCC ACC AAG GGC
                  398         407         416         425         434         443
```

FIGURE 1A

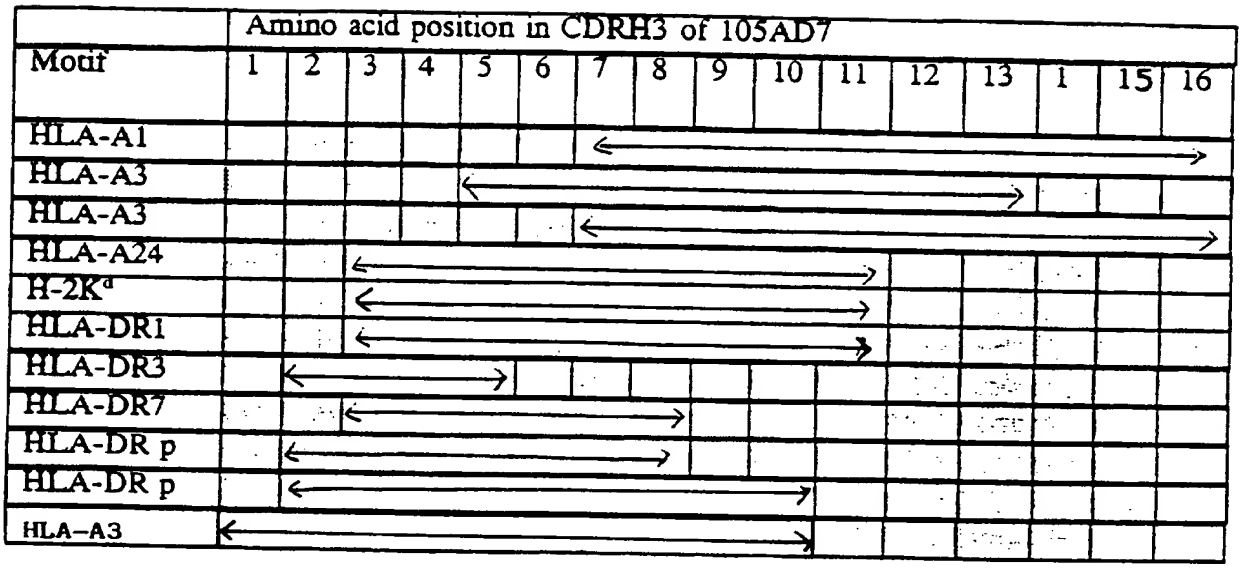
```
Frame 1   G   I   R   L   D   I   E   I   T   Q   S   P   S   S   L   S
        GGA ATT CGG CTT GAC ATC GAG ATC ACC CAG TCT CCA TCC TCC CTG TCT
                  9          18          27          36          45

                                          <----- (CDR K1) ----------
  A   S   V   G   D   R   V   R   I   T   C   R   A   S   Q   D   I   S
GCA TCT GTA GGA GAC AGA GTC AGG ATC ACT TGC CGG GCA AGT CAG GAC ATT AGC
         57          66          75          84          93         102

---------------->
  S   F   L   N   W   Y   Q   H   K   P   G   K   A   P   K   L   L   I
TCC TTT TTA AAT TGG TAT CAG CAT AAA CCG GGG AAA GCC CCT AAG CTC CTG ATC
         111         120         129         138         147         156

    <----- (CDR K2) ---------->
  Y   A   A   S   I   L   Q   S   G   V   P   S   R   F   S   G   S   G
TAT GCG GCA TCC ATA TTA CAA AGT GGG GTC CCA TCA AGG TTC AGT GGC AGT GGA
         165         174         183         192         201         210

  S   G   T   D   F   T   L   T   I   T   I   L   Q   P   G   D   F   A
TCT GGG ACA GAT TTC ACT CTC ACC ATC ACC ATT CTG CAA CCT GGA GAT TTT GCA
         219         228         237         246         255         264

                  <---------- (CDR K3) ---------------->
  T   Y   Y   C   Q   Q   S   Y   K   T   P   P   S   F   G   Q   G   T
ACT TAC TAC TGT CAA CAG AGT TAC AAG ACC CCT CCT TCT TTT GGC CAG GGG ACC
         273         282         291         300         309         318

  K   L   K   T   N   E   L   W   L   H   T   V   F   I   S   H   L   M
AAG CTA AAG ACT AAC GAA CTG TGG CTG CAC ACT GTC TTC ATC TCG CAT CTG ATG
         327         336         345         354         363         372

  S   S   E   L   D   C   S
AGC AGT GAA CTG GAC TGC TCT G
         381         390
```

FIGURE 1B

Figure 2

Amino acid	V	L	Y	Y	D	F	W	S	G	Y	L	E	Y	F	A	Y

Figure 3.



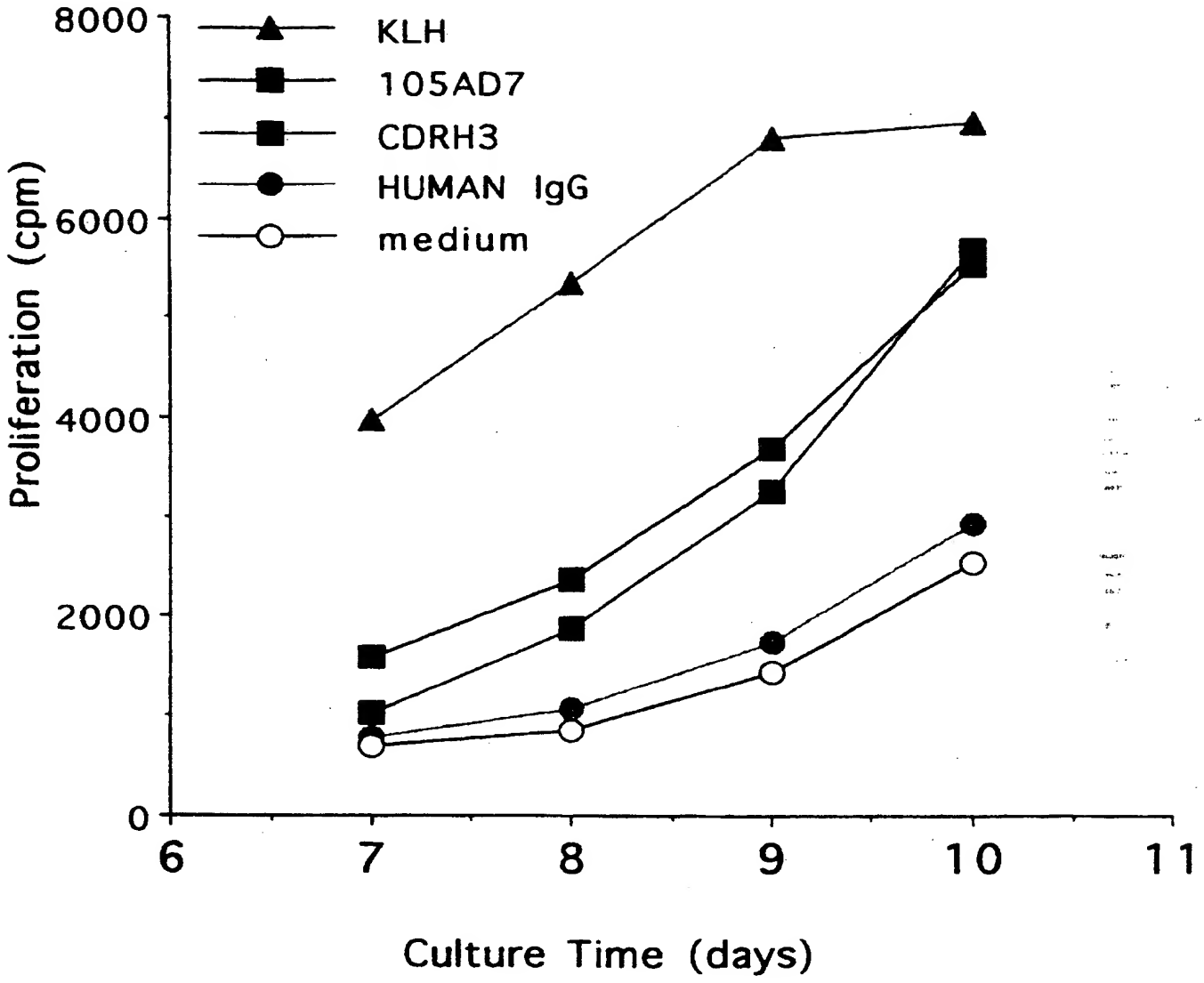


FIGURE 4

INTERNATIONAL SEARCH REPORT

Int. .onal Application No
PCT/GB 97/00591

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/13 C07K16/42 G01N33/53 A61K39/395 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 90 04415 A (XOMA CORP) 3 May 1990 see the whole document ---	1-21
X	CANCER RESEARCH, vol. 54, September 1995, pages 4837-4840, XP002036588 DURRANT ET AL.: "Enhanced cell-mediated tumor killing in patients immunized with human monoclonal antiidiotypic antibody 105AD7" cited in the application see the whole document --- -/--	1-21

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 August 1997	12. 08. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Hagenmaier, S

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BORREBAECK: "Antibody engineering" , OXFORD UNIVERSITY PRESS , 1995 XP002036589 Generation of antibody gene repertoires using the polymerase chain reaction (PCR), pages 56-61 see the whole document	1-21
A	US 5 314 996 A (WRIGHT JR GEORGE L) 24 May 1994 see column 12, line 4-68 see the whole document	1-21
A	WO 93 12232 A (DANA FARBER CANCER INST INC ;NEW ENGLAND DEACONNESS HOSPITA (US)) 24 June 1993 see the whole document	1-21
A	CANCER IMMUNOLOGY AND IMMUNOTHERAPY, vol. 38, 1 January 1994, pages 75-82, XP000568706 CHATTERJEE M B ET AL: "IDIOTYPIC ANTIBODY IMMUNOTHERAPY OF CANCER" see page 76, right column see the whole document	1-21
A	JOURNAL OF IMMUNOL., vol. 141, November 1988, pages 3227-3233, XP002036673 CAMPBELL ET AL.: "Immunotherapy of established murine B cell lymphoma" see the whole document	1-21
A	TRENDS IN BIOTECHNOLOGY, vol. 11, 1 February 1993, pages 42-44, XP000561907 HARRIS W J ET AL: "THERAPEUTIC ANTIBODIES - THE COMING OF AGE" see the whole document	1-21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 97/00591

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 19,21;12
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 19 and 21(all completely) and claim 12 (partially as far as an in vivo method is concerned) are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. onal Application No
PCT/GB 97/00591

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9004415 A	03-05-90	AT 130764 T	15-12-95
		AU 634181 B	18-02-93
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		JP 8503121 T	09-04-96

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